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Assessment of Eastern Massasauga Rattlesnakes (Sistrurus Catenatus) in Berrien County Historical Sites

Roshelle M. Hall
Andrews University, hall@andrews.edu

ABSTRACT

ASSESSMENT OF EASTERN MASSASAUGA RATTLESNAKES (*SISTRURUS CATENATUS*) IN BERRIEN COUNTY HISTORICAL SITES

by

Roshelle M. Hall

Chair: Daniel Gonzalez-Socoloske, Ph.D.

ABSTRACT OF GRADUATE STUDENT RESEARCH

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Title: ASSESSMENT OF EASTERN MASSASAUGA RATTLESNAKES (*SISTRURUS CATENATUS*) IN BERRIEN COUNTY HISTORICAL SITES

Name of researcher: Roshelle M. Hall

Name and degree of faculty chair: Daniel Gonzalez-Socoloske, Ph.D.

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The Eastern Massasauga rattlesnake (*Sistrurus catenatus*; EMR) is a small robust pit viper currently found in nine states and the province of Ontario, Canada. Wetland habitats have experienced significant destruction and fragmentation by humans; as a result, the current distribution of the EMR is a fraction of its historic distribution. For this reason, the EMR has been federally listed as threatened. In general, little is known about the current distribution of this rattlesnake (in the southwest corner of Michigan, the size of local populations or their stability and genetic diversity. Much of this knowledge is based upon historical data. My purpose was to update the available information on the current status in Berrien County and one Van Buren County site. This was done through presence/absence surveys, evaluation of potential threats at each site visited and genetic analysis at the haplotype level. Through my field surveys I confirmed presence of EMRs at 3 of the 5 historic locations surveyed. Current threats at these

sites include human encroachment, road traffic, and general health of the particular habitat. Despite the relatively small sample size and isolated populations in these counties, the haplotype diversity discovered appears to be high in comparison to the rest of their range.

Andrews University

College of Arts and Sciences

ASSESSMENT OF EASTERN MASSASAUGA RATTLESNAKES (*SISTRURUS CATENATUS*) IN BERRIEN COUNTY HISTORICAL SITES

A Thesis

Presented in Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Roshelle M. Hall

2019

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(*SISTRURUS CATENATUS*) IN BERRIEN COUNTY
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APPROVAL BY THE COMMITTEE:

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Daniel Gonzalez-Socoloske, Ph.D., Chair

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Peter J. Lyons, Ph.D.

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James L. Hayward, Ph.D. Date approved

To Kenley

My best friend and husband, I love

You ten times more than you could ever imagine.

To Mabel and Josh

The greatest kids in the world and best huggers.

Of course to my Precious Lord and Savior.

And to my Mom, I made it!

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LIST OF ABBREVIATIONS

BC	Berrien County
BLP	*
BNC	*
bp	base pair
BTF	*
CC	Cass County
CCAA	Candidate Conservation Agreement with Assurances
DLO	Date of Last Observation
DNR	Department of Natural Resources
DWP	*
EMR	Eastern Massasauga Rattlesnake
INB	*
LIL	*
MLB	*
MNFI	Michigan Natural Features Inventory
MNHD	Michigan Natural Heritage Database
mtDNA	mitochondrial Deoxyribo Nucleic Acid
ND2	NADH Dehydrogenase Subunit II
PCR	Polymerase Chain Reaction

PIT	Personal Identification Tag
SNC	Sarett Nature Center
SSA	Species Status Assessment
USFWS	United States Fish and Wildlife Services
VB	Van Buren County

*Full name not included due to EMR Research Protocol.

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This master's thesis would not have been possible without the contributions of numerous people. First and foremost is my Lord and Savior, Jesus Christ. Without Him, I could do nothing. With Him, everything is possible. I know it is only through the strength and love of Christ that I made it through all of my education and trials. It was the amazing faith of my grandparents and mother that I learned how awesome God is!

Where would I be without my intelligent and caring husband, Kenley? He has served as not only my constant companion, but also the one who knew I could do this even when I completely doubted myself. Mabel and Josh my awesome kids: you energize me and have given me courage and the encouragement and love to keep going. You always remind me that it's my turn, thank you! Mom—you have always supported me and encouraged me in the pursuit of my educational goals and without that important support I do not know where I would be right now, thank you. Dad, thank you for encouraging me, always telling me I can do it and being excited for me even when my research seemed crazy. I have the most amazing family, you are all such patient and loving people when I need it most.

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CHAPTER 1

INTRODUCTION

The family Viperidae are venomous snakes found in both the New and Old World. All Viperidae have long, hinged fangs while some, known as pit vipers, also have a special organ, known as a pit, in between the nostrils and eyes that detect differences in temperature. Two genera of Viperidae have evolved a warning system of specialized keratin called rattles on their tails. These rattlesnakes are restricted to the new world. Sistrurus and Crotalus are the two genera of rattlesnakes found in various habitats throughout North America. *Sistrurus* is distinctively different from *Crotalus* in overall size and scalation pattern (Gloyd, 1974). The scales on the head of *Crotalus* are much smaller and more numerous than on *Sistrurus. Sistrurus* individuals typically have nine larger head scales or plates (Gloyd, 1974; Klauber, 1972). The genus *Crotalus* contains at least 29 extant species, while *Sistrurus* contains only four species. My research focused on *Sistrurus,* found only in North America and parts of Mexico. Until recently these snakes were split into three species, the massasauga (*Sistrurus catenatus*), the pygmy (*Sistrurus miliarius*), and the mexican pygmy (*Sistrurus ravus*) rattlesnakes (Murphy, Fu, Lathrop, Feltham, & Kovac, 2002) (Figure 1). All of these pit vipers are relatively small, robust snakes. Adult snout vent lengths are 60-75 cm, 38-60 cm, and 40-65 cm for massasaugas, pygmy rattlesnakes, and mexican pygmy rattlesnakes respectively. Pygmy rattlesnakes have smaller rattles and longer tails in relation to overall length than

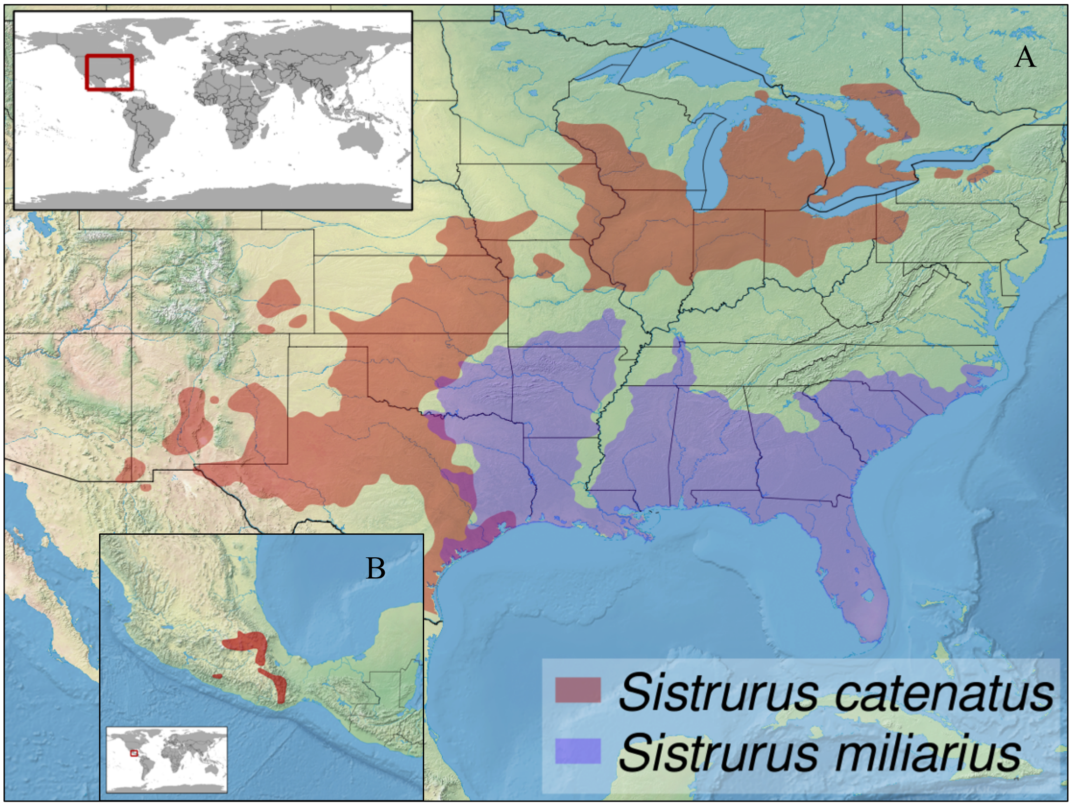

Figure 1. Distribution of pygmy, Mexican pygmy and massasauga rattlesnakes. A. The purple area depicts the range of the pygmy rattlesnake (*Sistrurus milliarius*), while the brown area depicts the *Sistrurus* species' (western massasauga-*Sistrurus tergeminus*, desert massasauga-*Sistrurus t. edwardsii* and eastern massasauga-*Sistrurus catenatus*). B. Depicts range of mexican pygmy rattlesnake-*Sistrurus ravus* (commons.wikimedia.org).

massasaugas (Gloyd, 1974). Pygmys have one lateral spot, whereas massasaugas have three (Gloyd, 1974).

Overview of the Massasauga

The wide, patchy range of *Sistrurus catenatus* contain parts of Ontario, Canada and the Great plains of the United States, including Texas and even parts of Arizona (Figure 2). Massasaugas have lost much of their habitat due to fragmentation and anthropogenic disturbance (Hobert, Montgomery, & Mackessy, 2004; Anderson, Gibbs, Douglas, & Holycross, 2009; Johnson, Gibbs, Bell, & Shoemaker, 2016). Gloyd (1974)

Figure 2. Distribution of Desert, Western and Eastern Massasaugas. Each species is depicted by color. Green area depicts eastern massasauga (*Sistrurus catenatus*) range, the blue area depicts western massasauga (*Sistrurus tergeminus tergeminus*) and the red area depicts desert massasauga (*Sistrurus t. edwardsii*) range (Mackessy, 2005).

extensively studied all *Sistrurus spp.* in the early 1940-50s. His research detailed some important variations in the overall appearance between the three *Sistrurus spp.* In general, massasaugas show a gradient in coloration with lighter populations in the western portion of the range and darker populations as one moves to the eastern portion. The massasauga species also inhabit slightly different niches within their individual ranges and vary in coloration and ventral scale count throughout their range (Gloyd, 1974; Klauber, 1972). The ventral scales of the western and desert massasaugas are very pale whereas the eastern massasaugas have blotchy and darker ventral coloration (Gloyd, 1974).

Each massasauga; desert, western and eastern are geographically separated and have evolutionarily divergent populations; in turn they have numerous intraspecific differences in physiology (Holycross & Douglas, 2006; Wooten & Gibbs, 2012). Previously the three massasauga snakes were considered to be a single species divided into three subspecies. Recently, through genetic research EMRs have been elevated to their own species (Kubatko, Gibbs, & Bloomquist, 2011; Gibbs et al., 2011; Ray et al., 2013). Eastern massasauga rattlesnakes (*Sistrurus catenatus*; EMR) are currently found in nine states and the province of Ontario, Canada (Gibbs, Murphy, & Chiucchi, 2011; Szymanski, 2015). Desert (*Sistrurus tergeminus edwardsii*) and western massasaugas (*Sistrurus tergeminus tergeminus*) are found further south and west as pictured in Figure 2 (Klauber, 1972; Wastell & Mackessy, 2011). Desert and western massasaugas are considered subspecies of one another (Anderson et al., 2009; Ray et al., 2013).

Eastern Massasauga Rattlesnake Life History

Eastern massasauga rattlesnakes are secretive rattlesnakes endemic to the Great Lakes region. They are an important species in the ecological community, as massasaguas are both predator and prey. *S. catenatus* feed mainly on small rodents and, in turn, are eaten by large raptors and larger snakes (Keenlyne & Beer, 1973; Tetzlaff, Ravesi, Parker, Forzley, & Kingsbury, 2015). Unlike many other species of rattlesnakes that inhabit arid areas, EMRs inhabit shallow wetlands with few trees (marshes, fens, and bogs) and associated woodlands (Giovanni, Hileman, Jaeger, & King, 2009; Harvey & Weatherhead, 2010; DeGregorio, Putman, & Kingsbury, 2011; Bailey, Campa, Bissell, & Harrison, 2012). These habitats have experienced significant destruction by humans and as a result, the current distribution of EMRs are a fraction of their historic distribution (Szymanski et al., 2015). Of the 581 historic populations, 121 (20%) are listed as status unknown and only 267 (45%) are known to still be extant (Szymanski et al., 2015). In addition, 154 (40%) of the presumably extant populations (known extant plus unknown status, n=388) are likely quasi-extirpated (less than 25 adult females; see Figure 3) (Szymanski et al., 2015).

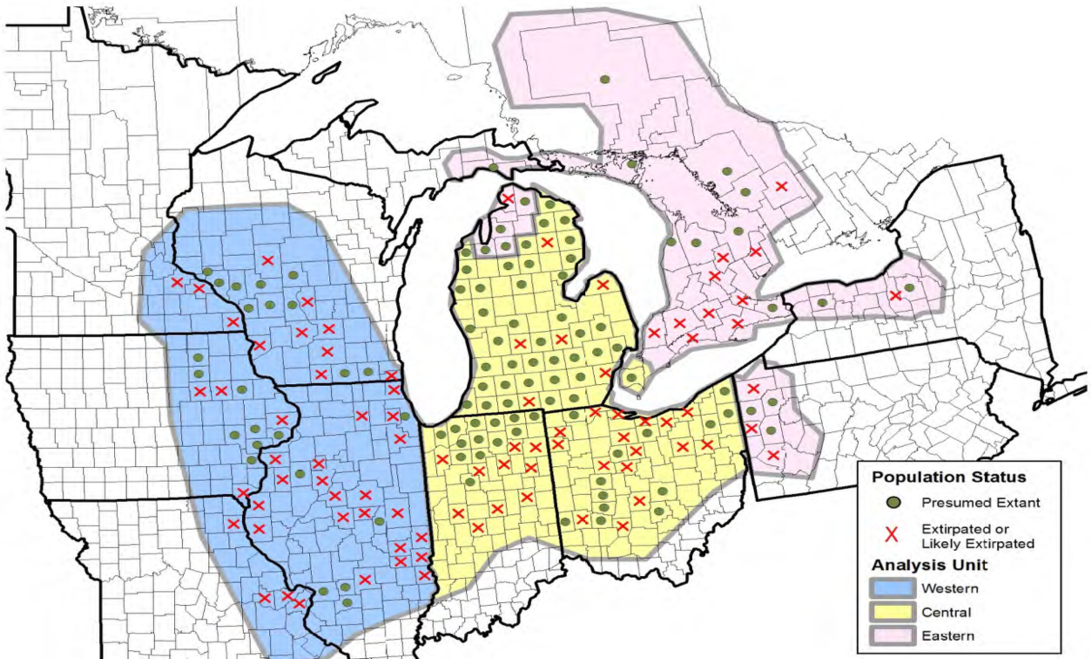

Figure 3. Eastern massasauga rattlesnake distribution. The geographical distribution of presumed extant (extant and unknown status) and extirpated EMR populations. Depicting counties of historical populations both extirpated and extant. (Szymanski et al., 2015).

Eastern massasauga rattlesnakes are listed as endangered or threatened in every state and now federally listed as threatened (Szymanski et al., 2015; United States Fish and Wildlife Services [USFWS], 2016). Michigan is known as the last stronghold of EMRs because there are more intact habitats and populations than the other eight states with historical populations (Baker, Davis, Anthonysamy, & Dreslik, 2018; Johnson et al, 2016; Syzmanski et al, 2015). However, even in Michigan EMRs are a rarity, with populations thought to be decreasing due to habitat loss, fragmentation, persecution and predation (Moore & Gillingham, 2006; Jones et al., 2012). Researchers have studied behavioral ecology, microhabitat use, thermoregulation patterns, responses to human management, eating habits of neonates and adults, road kills, brood sizes, and male and female activity and body size (Cross et al., 2015; DeGregorio et al., 2011; Dovčiak, Osborne, Patrick, & Gibbs, 2013; Durblan, 2006; Harvey & Weatherhead, 2010; Keenlyne & Beer, 1973; Robillard & Johnson, 2015; Shepard, Kuhns, Dreslik, & Phillips, 2008; Tetzlaff, Ravesi et al., 2015). Although this species has been extensively studied, many basic questions still need to be answered.

Eastern massasauga rattlesnakes have been extirpated from much of their range in Michigan and even more so in the other states where they were once abundant (Jones et al., 2012; Szymanski et al., 2015; USFWS, 2016). Habitat assessments have been accomplished throughout the EMR's range to discover the types of habitat, the typical home range, and even much of their seasonal habitat use (Harvey & Weatherhead, 2006b; Jones et al., 2012; Moore & Gillingham, 2006). Cass County (CC) and other eastern and northern counties in Michigan have been studied, but no research or systematic studies have occurred in Berrien County (BC).

Introduction to Research

The objectives of this study were to: (1) determine continued EMR presence using a variety of validated and novel survey techniques at selected historic population sites in BC, (2) assess the remaining EMR habitat (general threats and conservation efforts) occurring at each visited historic population site in BC, and (3) determine genetic relatedness of BC EMR with other populations.

In Chapter 2, I discuss the methods and findings from my visual surveys at five sites within BC. Although Michigan is considered its last stronghold, even here EMRs are a rarity, with populations becoming fragmented and therefore decreasing in numbers. In general, little is known about the current abundance of this rattlesnake therefore, determining the actual presence of EMRs at any of the historical sites in BC is important to their survival. This chapter provides information on the variety of survey techniques, discusses the validity of these techniques and, provides basic information about each EMR detected. Because EMRs are decreasing in populations and numbers, this information can aid the state of Michigan in their protection of the species.

Chapter 3 assesses the conservation efforts and threats at each site surveyed. The shallow wetlands habitats here in BC are owned and managed by various individuals and organizations. Each site had its own management process dependent upon the owners. Many of these properties experienced or continue to experience significant destruction by humans. Some properties maintain natural habitat health using controlled burns, cutting of overgrowth, mowing and careful herbicide spraying on a yearly basis, while others do very little to maintain the natural state of their property. My goal was to provide a general habitat assessment for each property and aid in protecting many species, not just the EMR.

Chapter 4 documents the results of my genetic analysis of any EMRs captured during this study. I took samples of EMR blood and sheds discovered at each of my study sites to further aid understanding of this cryptic species. The Association of Zoos and Aquariums is currently involved in breeding programs and attempting to understand the genetic relatedness of various populations of EMRs. No genetic testing had occurred in BC; therefore, adding this aspect to my research was also of vital importance. I evaluated the haplotypes in BC compared to other populations in Michigan and nearby states to further clarify geographic boundaries and genetic makeup of local EMRs (Ray et al., 2013).

My final chapter (Chapter 5) presents a summary of major points and recommendations for future research in BC for the EMR. Ultimately, this study provides scientifically based information that can be used by governing agencies and land managers to better conserve this threatened species.

CHAPTER 2

EASTERN MASSASAUGA RATTLESNAKE PRESENCE IN BERRIEN COUNTY HISTORICAL SITES

Introduction

The EMR is extremely cryptic and difficult to survey. This makes monitoring this species and recognizing population trends very difficult for wildlife managers. The EMR is currently found in nine states and the province of Ontario, Canada (Gibbs et al., 2011; Hileman et al., 2017; Szymanski et al., 2015).

Although the EMR is still found in nine states, Michigan has more intact populations than any other state (Shoemaker & Gibbs, 2010; Missouri Department of Conservation, 2004; Szymanski et al., 2015). Southern Michigan also has the most continuous pattern of counties with EMR populations, yet little is known about most of these populations (Figure 4). Due to significant destruction of habitat and decline of EMR numbers, USFWS federally listed EMR as threatened species in 2016 (USFWS, 2016).

According to the currently accepted standard EMR survey protocol (Casper et al., 2001), a population cannot be considered extirpated (locally extinct) unless surveys have yielded zero sightings for 15 consecutive years. Very few land managers have the time or resources to properly determine the status of EMR at various locations. Until 2015, no formal research had occurred in BC even though Southern Michigan is considered a stronghold within the state. A CC site is the furthest south in Michigan where extensive

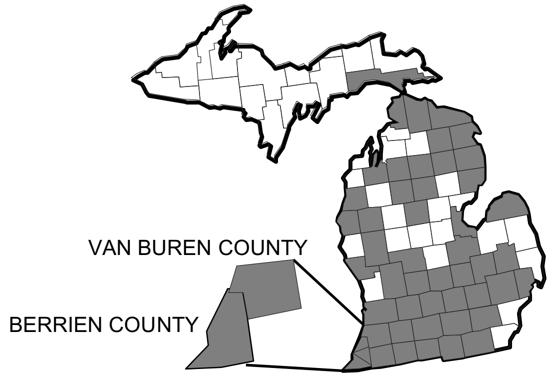

Figure 4. Map of counties with eastern massasauga populations. Map of Michigan, all counties shaded in gray have at least one population of *Sistrurus catenatus.* The two enlarged counties depict the research focus.

research has occurred (Hileman, Bradke, Delaney, & King, 2015a; Hileman, Kapfer, Muehlfeld, & Giovanni, 2015b; Hileman et al., 2017; Hileman, Vecchiet, King, & Faust, 2012). Before this research project all current information in BC was based upon anecdotal sightings.

Three of the eight locations in BC are currently listed as "unknown status" or "likely extirpated," with last dates of observation ranging from 1988-1993 (see Table 1). Currently there are no population estimates for any of the historical sites. Even the more recent dates were incidental findings of one or two EMR and no additional data was gathered. The first objective of the research was to determine the presence/absence of EMR at any historical sites to which I gained access (ambiguity about the locations follows recommended procedure by Michigan Department of Natural Resources (DNR) protocol to avoid poaching of this threatened species). The overall aim of this research is to strengthen the understanding of the extant populations of EMR's in BC, updating current information and status of select historical sites.

Table 1

Michigan Natural Features Inventory (MNFI) List of site owners with dates of EMR Observations[a]

Owners of Location	Abbrev.	1998 SSA Status	1998 SSA Trend	1998 DLO[b]	2014 SSA Status	2014 DLO
SW MI Land Conservancy	BTF	Extant	Vulnerable	1993	Unknown	1993
BC Parks	INB	Extant	Vulnerable	1992	Extant	2013
Buchanan Twp.	MLB	Extant	Vulnerable	1991	Unknown	1991
Little Indian Lake	LIL	Extant	Vulnerable	1988	Likely Extirpated	1988
Chikaming Open Lands	DWP	Extant	Vulnerable	1987	Extant	2002
Sarett Property	SNC	Extant	Secure	1995	Extant	2014
MI Nature Assoc.	BNC	N/I[c]	N/I	N/I	Extant	2013
Black Lake	BLP	N/I	N/I	N/I	Extant	2014

[a] Table summarizes the Michigan Natural Heritage Database (MNHD) of rare species and community occurrences from Berrien & Van Buren counties.

[b] Date of Last Observation.

[c] No Information.

[d] These data are very important and any research on EMR's at sites in Michigan need to be reported to the MNHD to aid with the species survival plan.

Methodology

I contacted each owner in Table 1 and determined five sites in BC to survey for EMR. Once I established which sites to survey, yearly permits were obtained from Michigan DNR and in 2017 a Federal permit was applied for (and received) given that EMR was now considered a threatened species. With permits in hand, permission granted from property owners, and proper training received, surveys began.

The same select sites were surveyed in 2016 and 2017 through standard visual surveys. My surveys occurred between 15 April and 20 October for a total of 33 days in 2016. In 2017 surveys were between 2 April and 24 September for a total of 20 days. Each survey began and ended with recording air temperature, ground temperature, wind and cloud cover. I recorded time spent actively searching for each surveyor. Visual surveys included walking through grass, cattails, near hummocks of prairie fens, in and around all shrubs and into the woodland areas at each property, inspecting for snakes (Casper et al., 2001). In addition, 61 cm by 61 cm wooden boards and carpet remnants, were utilized at two of the sites to facilitate detection (Bartman, Kudla, Bradke, Otieno, & Moore, 2016; Giovanni et al., 2009; Hileman et al., 2015b). I placed two boards and two carpet remnants in random locations at the sites DWP and BNC. Past studies have shown that artificial cover boards provide habitat enhancement and often are used for thermoregulation or serve as a refuge from predators by various reptiles and amphibians (Glowacki & Grundel, 2005; Ed McCuisten, personal communication). Because the wood and carpet are frequently used by reptiles, I hoped they would aid in finding EMR. During my surveys I checked both on top of and under these pieces for EMR or other reptiles.

Once detected, EMR were initially captured using snake tongs. Captured EMR were secured within a cloth bag, a knot was tied near the opening and the bagged snake was temporarily transported within a properly secured plastic bucket with adequate ventilation. I processed each EMR onsite in an open location to avoid escapes. Snake processing included taking body measurements, sexing, and collection of blood for DNA analysis. Determination of sex in most of the captured snakes entailed counting subcaudal scales, as shown in Figure 5A (Dreslik, 2005; Klauber, 1972). Male EMRs have more subcaudal scales than females, especially in relation to length of their tail. Females have 19-29 scales and males have 25-33 subcaudal scales (Klauber, 1972). Finally, a small 12 mm AVID personal identification tag (PIT) was inserted sub-dermally to simplify identification if an individual snake was captured again. Any dead snakes could also be identified if previously captured in any subsequent year. All PITs were placed on lower left side about two-thirds back from the head of each EMR. Personal identification tags are the standard identification method used in wildlife studies since the mid-1980s and have resulted in very few mortalities across thousands of studies and taxa (Gibbons & Andrews, 2004). Neonates were not tagged with a PIT; rather they were branded following the research protocol used at the Edward Lowe Foundation in CC (Giovanni et al., 2009). To collect blood and insert the PITs, snakes were restrained using a clear plastic tube. "Tubing" is a common method used with venomous snakes in which the head and most of body is placed within a tube so the snake cannot turn around (Figure 5B).

Photographs of each snake were taken to help identify as well as document and verify each sighting via photographic voucher. Habitat photographs were also taken at each site and in each microhabitat for plant community identification and verification (Figure 5C) (Casper et al., 2001). A "squeeze box" (wooden box with a glass cover) (Figure 5D) was used to gently pin the snake down so that calibrated photographs were safely taken of the head and body, and accurate measurements taken (Bertram & Larsen, 2004). To measure the EMR in this fashion, I used a dry erase marker following the length of the snake then placed a string along that line and measured the string.

Figure 5. Photos of methodology descriptors. **A**. EMR subcaudal scales used to determine sex and take the blood sample by inserting needle above the rattles into subcaudal vein. Measuring from cloaca (note arrow) to rattle determines tail length (subtract this from total length to achieve SVL) of specimen. **B.** Tubing a snake. **C.** EMR in-situ depicting (crypsis). **D.** EMR in "Squeeze box" with plexi-glass lid.

Processed snakes were released at the location of capture. I followed a working protocol (see Appendix A) to ensure that all the necessary precautions were taken to keep the snakes and human handlers safe. Snakes were never held for longer than 24 hours for processing.

Due to the presence of fungal infections (*Chrysosporium* sp.) found in some EMR (Allender et al., 2011; Tetzlaff, Allender, Ravesi, Smith, & Kingsbury, 2015) and other possible infections and parasites that can be spread between individual snakes and sites, I practiced the recommended hygiene protocol outlined in Habitat Management Guidelines for Amphibians and Reptiles of the Midwestern United States 2nd Edition (Appendix A).

Results

Environmental data, total survey time, species of reptiles encountered, and date were recorded each survey day. During the two years of surveying, April to October in 2016 and April to September in 2017, my team spent a total of 612.9-person hours surveying and capturing 11 EMR (Table 2). Five of the eight historical locations were surveyed. Two sites, LIL and BLP were privately owned and the historical descriptions made it difficult to determine their exact locations. One site, Sarett Nature Center (SNC), refused to grant permission to survey for EMR. A total of 11 EMRs were captured from three of the five locations surveyed (Table 2). I did not confirm EMR presence on two of the historical sites based on the surveys.

Table 2 gives an overview of the total EMR detected at each site, sum of hours spent surveying and the average number of hours it took my team to confirm presence of massasaugas at three of the sites surveyed. I calculated the number of expected EMR based on the mean number of person-hours/EMR in the three sites where I did detect them. One of my sites is on the border of Berrien and Van Buren county. At this site, one EMR was a recapture. The average detection rate was 0.0262 EMR per survey hour. A mean of 38.2 person-hours were needed to detect one snake.

Table 2

Calculated Person-hours versus EMR during Surveys

Site	Total Hours[a]	Total Person Hours	Total EMR	EMR/PHR	PHR/EMR	Expected[b]
DWP	47.1	167.0	5	0.0299	33.3	4.4
INB	12.8	34.4	1	0.0291	34.4	0.9
BNC	71.1	256.7	5	0.0195	44.7	6.7
BTF	45.1	117.0	0			3.1
MLB	18.8	37.7	0			1.0
TOTAL	**195.0**	**612.8**	**11**	**MEAN**	**38.23**[c]	

[a] Columns depict personal hours then total hours with assistants surveying at each site. Next is number of EMR captured, the number of EMR per person hour and how many person-hours to detect each Massasauga. and finally, the calculated number of EMR expected due to the number of hours spent at that site
[b] The calculated number of EMR expected to be found at each site due to average number of person hours/EMR
[c] The final row shows the calculated average of hours it took to find an EMR using all data.

Discussion

Table 2 reveals the disparity of hours spent at two of the sites versus the other three. These two sites were more difficult to survey. One site was a natural kettle bog and much of this site was dangerous to traverse since peat moss in a bog is not typically firm enough for standing or walking. Eastern massasauga rattlesnakes are found in bogs and peat hummocks, especially during their active season (Johnson et al., 2016). The other site requires either a boat or a three-hour hike to enter the area where EMR can be found.

I was able to confirm presence of EMR at three of the five historical sites I evaluated. I located both male and female EMR as well as younger EMR at two separate sites. (Figure 6A and 6B). This suggests the presences of actively reproducing populations. In 2016 a gravid EMR was found at two of these sites. From the survey findings I was able to update date of last observation (DLO) on three of the five locations. One of the sites (BNC) bleeds into Van Buren County (see Table 3).

Figure 6. Examples of EMR found. **A.** EMR in partial shed (very likely a reason to be more aggressive.) note opaque eyes. **B.** Youngest EMR encountered, was basking on a log.

Table 3

Updated Version of MNFI-Species Status Assessment (SSA) Chart[a]

Location Abbreviation	1998 SSA Status	1998 SSA Trend	1998 DLO	2014 SSA Status	2014 DLO	My Study
BTF	Extant	Vulnerable	1993	Unknown	1993	0 (exp. 3.1)[b]
INB	Extant	Vulnerable	1992	Extant	2013	2017
MLB	Extant	Vulnerable	1991	Unknown	1991	0 (exp. 1.0)
LIL	Extant	Vulnerable	1988	Likely Extirpated	1988	No Surveys
DWP	Extant	Vulnerable	1987	Extant	2002	2017
SNC	Extant	Secure	1995	Extant	2014	No Surveys
BNC	N/I	N/I	N/I	Extant	2013	2017*
BLP	N/I	N/I	N/I	Extant	2014	No Surveys

[a] Table summarizes the MNHD and includes information from my 2016-2017 survey efforts.
* Denotes the site that bleeds into Van Buren County.
[b] The calculated expected number of EMR I should have detected according to Table 2.

Although this research was carried out for only two seasons, not finding evidence of EMR at two of these historical sites was troubling, especially for BTF, where I expected to find multiple EMR based on survey effort (Table 2). The last EMR seen at these two sites was over 24 years ago. However, more surveying over numerous seasons needs to be done in order to determine if the EMR population on these two sites can be considered extirpated (Casper et al., 2001; Glowacki & Grundel, 2005; Bradke et al., 2018). This would require a large investment of time. My research indicates that 38.23 person-hours are needed to detect one snake. I feel the number of person hours is high for these sites as a result of overgrowth of habitat, unknown areas of possible activity for the EMR, and the very cryptic nature of the snake itself (Marshall, Manning, & Kingsbury, 2006), however this value can serve as a rough indicator of the amount of survey effort needed to detect EMR in BC.

Recommendations

Berrien County needs to continue research at these sites. Due to the cryptic nature of EMR, in order to determine actual extirpation and population estimates, this study should be conducted for a minimum of ten years, and while this occurs protection of the habitats should continue. Long term demographic information is crucial to access and guide the conservation process of any threatened species (Bradke et al., 2018; Michigan DNR, 2016). Any EMR sighted should also be a catalyst for ecosystem restoration (Bailey et al., 2012; Casper et al., 2001).

As the public becomes educated on the existence of EMR, I have also learned of additional sites with EMR. Sightings at some of these locations have been validated and research should be expanded to these areas. I plan to continue searching in BC for EMR at known historical sites as well as possible new sites. Use of groups of experienced EMR researchers to visually survey at each site (a bio-blitze), will increase the likelihood of determining presence or absence in a shorter time span.

CHAPTER 3

EASTERN MASSASAUGA RATTLESNAKE HABITAT ASSESSMENT IN BERRIEN COUNTY HISTORICAL SITES

Introduction

Because EMR are associated with specific habitats, habitat characterization is a critical step to assess the status of the species. While my main objective is to characterize the status of the remaining EMR populations in BC, characterizing the microhabitats in which they are found and measuring the size and overall quality of those habitats is important as well. Habitat use and preference for the EMR is variable over its geographic distribution, although this species typically is found in wetlands, upper woodlands, and prairie fens and has specific hibernaculum needs (Johnson et al., 2000; Harvey & Weatherhead, 2006a).

Wetlands in the US are being fragmented and destroyed as a result of both natural and anthropogenic causes. Climate change, vegetative succession and invasive plant species encroach on suitable EMR home ranges. In addition, hydrological changes, agriculture, roads and other human impacts all affect the types of microhabitats required by EMR (Dovčiak et al., 2013; Durbian, 2006; Robillard & Johnson, 2015; Seigel, Sheil, & Doody, 1998; Shepard, Dreslik, Jellen., & Phillips, 2008; Shepard, Kuhns et al., 2008). Eastern massasauga rattlesnakes appear to be very sensitive to changes and recover slowly from population impacts and therefore can be considered a sentinel species.

Invasive plant species, monocultures, invasive woody plants and human intrusion all change the overall habitat health they need to survive. The wetland areas that EMR inhabit are also home to many important plant species and other herpetofauna. These include sundews (*Drosera spp.*), purple milkweed (*Asclepias purpurascens*), kirtland's snake *(Clonophis kirtlandii),* woodland turtles, salamanders and numerous frogs and toads (Slaughter, Hyde, Cuthrell, Lee, & Norris, 2013). As we learn how to assist in saving the populations of EMRs, this in turn will assist in the protection of other important species that share the same habitats.

The current status in BC of the wetland habitats where EMR have historically lived needs to be updated. The overall aim of this research is to strengthen the understanding of the historical properties that included extant populations of EMR in BC, and updating current status of those select historical sites.

Habitat Assessment

Eastern massasauga rattlesnakes have been located in various microhabitats including wet prairie (Seigel et al., 1998), fens and sedge meadows (Johnson & Leopold, 1998; Kingsbury, 1996; Kingsbury, Marshall, & Manning, 2003), peatlands (Johnson & Leopold, 1998), coniferous forests (Weatherhead & Prior, 1992), sedge meadows, and old fields (Reinert & Kodrich, 1982). At least three reasons account for this habitat diversity: 1) regional variation of habitats; 2) seasonal habitat shifts of EMR, with a preference wet prairies, fens, and sedge meadows in the spring and fall, and a preference for drier habitats in the summer (Reinert & Kodrich, 1982; Seigel, 1986; Johnson & Leopold, 1998); and 3) "some of this diversity is just a matter of semantics, as various authors and researchers use terms differently" (Johnson, 2000, p. 1). Regardless of these regional, seasonal, and semantic differences there are several common attributes of EMR habitats.

Open Canopy

For most reptiles, thermoregulation plays a primary role in habitat selection (Kingsbury, 1999; Huey and Stevenson, 1979). A primary factor in thermoregulation is the availability of open canopy (DeGregorio et al., 2011; Moore & Gillingham, 2006). However, numerous studies suggest that EMR will use coniferous forest or forest edge, adjacent to open-canopy as a foraging space (Weatherhead & Prior, 1992; Johnson & Leopold, 1998).

For EMRs the thermoregulation needs of gravid females are greater than those of their male counterparts or non-gravid females (Reinert & Kodrich, 1982; Johnson & Leopold, 1998). Because of their need for higher body temperatures, gravid females generally exhibit more "above-ground basking behavior" (Reinert & Kodrich, 1982; Moore & Gillingham, 2006).

Dense Ground Cover

Eastern massasauga rattlesnakes, like many snakes and lizards employ behaviors or actual visual camouflage to prevent detection (Schwarzkopf & Shine, 1992; Parent & Weatherhead, 2000). While above ground, EMR utilize this cryptic behavior in dense ground cover to avoid detection by potential predators (Casper et al. 2001, Melville & Swain, 2007; Parent & Weatherhead, 2000). Thus, for detection avoidance dense groundcover is essential near basking sites.

Proximity to Water

Another commonality of a healthy EMR habitat is the proximity to water (Missouri: Seigel, 1986; Wisconsin: King, 1999; Indiana: Minton, 1972; Kingsbury, 1996, 1999; Pennsylvania: Maple & Orr, 1968; Reinert & Kodrich, 1982; New York: Johnson & Leopold, 1998). The connection of EMR to wetlands is intriguing since EMR are not even semi-aquatic. Yet, most EMR move to the wetlands in the fall no matter where they were found during the active season. This move to wetlands in the fall is typically connected to hibernation. Wetland areas offer saturated soil. Additionally, they provide crayfish burrows which are used by EMRs for hibernation (Kingsbury, 1999; Maple & Orr, 1968; Seigel, 1986). Sphagnum hummocks (Johnson & Leopold, 1998) are also used for hibernation.

Hibernaculum

Hibernacula for EMR varies by geographic location. Although they are primarily reported to hibernate in crayfish holes they are also known to use small mammal burrows (Vogt, 1981; McCumber & Hay, 2000; VanDeWalle, 2005). Numerous issues can alter hibernacula, such as successional changes in vegetation and desiccation of soil moisture. According to Johnson and Leopold (1998) in peatlands EMR typically burrow into moss and shrub hummocks. Successional changes in vegetation can cause hummocks to level out and the hibernaculum opportunities they offer to vanish. Additionally, EMR most often use crayfish burrows for overwintering. The succession of habitats by shrubs and trees often makes conditions less favorable for crayfish leaving fewer burrows for EMR. When this occurs, EMR have been known to use tree roots for hibernaculum.

Size and Connectivity

Although habitat size is important for the health and multiplication of EMR, even more important is the connectivity of the habitat. As previously noted EMR make seasonal habitat shifts generally to wet prairies and fens and sedge meadows in the spring and fall, and to drier habitats in the summer. Isolated habitats due to fragmentation can prevent EMR from getting to an open canopy basking site during their active season, isolate them from wetland hibernaculum sites, or cause risk to potential danger such as crossing roads or inhospitable gaps in an effort to reach a particular seasonal habitat (Johnson et al., 2000).

Potential Threats

The EMR is listed as endangered or threatened in every state and is now federally listed as threatened (USFWS, 2016; Szymanski et al., 2015). Researchers believe Michigan has more intact habitats and populations than the other eight states with historical populations. Even in Michigan, however, EMRs are a rarity, with populations thought to be decreasing due to habitat loss, anthropological threats, and a lack of clear conservation efforts.

Road Mortality

Like most snakes, one of the EMR greatest threats is human related mortality. Studies tracking radio tagged EMR have shown that 47% of the mortality of these EMR was due to road mortality (Weatherhead & Prior, 1992; Dreslik, 2005). Shepard, Dreslik et al. (2008) also reported that road mortality was a high potential threat at their Illinois study site. In 2014 a steward of a local preserve in BC found an EMR dead in the road at a Chikaming Open Lands preserve (Ryan Postema, personal communication).

Poaching

Not only was road mortality listed as a high potential threat at their Illinois site, but Shepard, Dreslik et al. (2008) also listed poaching of EMR for the pet trade as another significant potential threat. Unfortunately, EMR are “a valuable commodity by hobbyists and by the poachers who hunt for them.” (Johnson et al., 2000; Szymanski et al., 2015) Poachers often utilize EMR literature to identify the best potential spots for hunting EMR.

Exposure to Humans

Parks and nature preserves play a crucial role in the protection of natural habitats. However, they also often provide opportunities for public recreational activities such as hiking, biking, camping or off-road vehicle use. These activities can create unintended consequences for the habitat and the populations of EMR on that site (Smeenk et al., 2016). Snake fungal disease can be detrimental to populations of snakes and researchers do not know for sure how this fungus is transmitted. However, humans traveling between different sites where EMR’s reside could carry this disease on shoes or other equipment moved between recreational sites (Allender et al., 2011). Parent and Weatherhead (2000) argue that more research needs to be done to determine if there is a detrimental effect of human activity on EMR. One study found that both road mortalities and management actions make up the majority of human-related deaths (Jones et al., 2012). Nearby housing or agricultural land is another aspect of human exposure that can pose a threat to EMR.

Habitat Destruction-Habitat Succession

For most EMR populations, habitat destruction is the primary reason that EMR are endangered (Weller & Oldham, 1993; Szymanski et al. 2015). Woodland plant encroachment (Wright, 1941; Johnson & Leopold, 1998), a loss of ground cover (Casper et al., 2001; Melville & Swain, 2007), and fragmentation (Johnson et al., 2000; Szymanski et al., 2015) are the main issues causing habitat destruction. However, other studies (Gibbons et al., 2000) have shown supplementary factors including, but not limited to "environmental contaminants, commercial exploitation, coastal development, fire suppression, river and stream modification, and wetland degradation" (Weller & Oldham, 1993) that also cause habitat destruction for EMRs. When habitat fragmentation occurs, genetic richness often decreases. Loss of genetic resources for the EMR can be considered a threat to their overall, long-term survival (Baker, Anthonysamy et al., 2018).

Conservation Strategy

As noted under potential threats common habitat management practices can have unintended effects on habitat loss and EMR mortality. However, proper habitat management principles are also essential to protecting the EMR habitat. One study noted that correct management and lack of human encroachment increased EMR survivorship in that area (Bailey, Campa, Harrison, & Bissell, 2011).

Research was done looking at habitat management practices at each site. Included in the criteria are the, Candidate Conservation Agreement with Assurances (CCAA) for the EMR in Michigan (USFWS, 2019). Two of the research sites have signed agreements to work under the regulations of the CCAA.

Detailed Management Plan

A successful habitat management plan must have specific and easy to understand goals (Johnson et al., 2000). These goals should include but not be limited to addressing issues related to habitat complexities and fragmentation, keeping the site in its most natural state, management of water levels and hibernaculum management. Additionally, habitat management for EMR should also include the habitat needs of small mammals and crayfish, both of which are important to EMR as prey and burrow makers. Tools available for habitat management include, controlled burns, cutting/mowing/bush-hogging, changing the water table, herbicides, or any combination of these practices (Johnson et al., 2000; Szymanski et al., 2015).

Habitat Complexities

It is essential that land managers think in terms of habitat complexities and do not merely think in terms of isolated patches. Eastern massasauga rattlesnakes make seasonal habitat shifts. If habitats are not connected to each other EMR may not be able migrate from one to another or need take risks attempting seasonal habitat shifts. Habitat loss caused by fragmentation is a key issue in the decrease of EMR populations. It is essential that land managers address fragmentation either through restoration or by developing safe corridors for EMR to move between the isolated fragments (Beier & Noss, 1998; Harrison, 1992; Gates & Gysel, 1978; Andren & Angelstam, 1988; Colley, Lougheed, Otterbein, & Litzgus, 2017). Considering flora and fauna at each site also is important in maintaining habitat complexity. This includes understanding important plant species that need to be protected and other animal species such as turtles, small mammals, other snakes and even certain insects important to the natural state of the various microhabitats within the property.

Management to Maintain Natural State

A central component of effective land management strategies for EMR is to keep the wet prairies; fens and sedge meadows, peatlands, woodlands, sedge meadows and old fields in their most natural state. Natural disturbance processes have been disrupted because, wetlands have become fragmented and surrounded by human controlled lands such as agriculture, housing developments and roadways. This disruption has caused habitat and vegetative composition to change, often leading to succession or unnatural drought or flooding (CCAA). Often controlled burns, careful herbicide application, hydro-axing and various methods of cultivation can all aid in managing EMR land and bringing it back to a more natural state (CCAA). Durbian (2006) has shown, however, that two very common habitat management practices, mowing with blades at 10-15 cm from the ground and summer burning, have contributed to "substantial EMR mortality (p. 332)." It is important to carefully monitor how and when each management practice occurs so that minimal loss of life to any threatened or endangered animal occurs.

Management of Water Levels

Improper water level management that leads to fluctuating water levels can be catastrophic to EMR. In particular, lowering water levels during hibernation can have disastrous consequences. These can include but are not limited to exposure to sub-freezing temperatures (Carpenter, 1953; Maple & Orr, 1968); dehydration (Costanzo, 1989); the loss of lipids and liver-stored nutrients that protect against desiccation

(Roberts & Lillywhite, 1980; Graves, Duvall, King, Lindstedt, & Gern, 1986) and provide energy for reproduction when EMR emerge in the spring.

Conversely, studies show that there are no detrimental effects of raising water levels during hibernation. One reason for this is that during hibernation EMR can go without air for an extended period of time. There are two other important factors to consider in water level management. First, lowering water levels can increase plant succession. Second, either lowering or raising the water table can affect the crayfish population.

Hibernaculum Management

The most important part of hibernaculum management is the realization that even minimal alteration of the hibernacula must be avoided. Any loss of hibernacula will have a dramatic impact on the entire population. Thus, identifying and protecting the hibernacula is crucial for EMR conservation efforts.

Methodology

To determine which sites to study in BC I used the MNFI of rare species and community occurrences from BC and adapted the information into Table 4. Once verified, I wrote to each organization to gain access to the individual sites. With agreements between Andrews University Department of Biology, the various owners, and proper insurance, research began. Of the eight known historical sites listed in Table 4, two were historically ambiguous in regards to location. The six other sites were owned by conservation organizations, a local township, BC, and a nature center; I sent letters to each of these owners. The local nature center chose not to be involved in the research, but

Table 4

Species Status Assessment Property List for BC

Owners of Location	Abbreviation
SW MI Land Conservancy	BTF
BC Parks	INB
Buchanan Township	MLB
Unknown Location	LIL
Chikaming Open Lands	DWP
Sarett Property	SNC
MI Nature Association	BNC
Unknown Location	BLP

the other five site owners decided to partner with AU and I concentrated my efforts at those five sites.

Through the use of county maps and assistance of site owners I attempted to survey all of the property available to the EMR. During my research, I noted invasive species, plants listed as special concern or threatened, whether or not canopy was closing in the open prairie habitats and general hydrological health of each site. I also looked for signs of anthropogenic threats such as excessive garbage and litter, bullet casings, poaching evidence and road traffic.

An assessment rubric was developed to determine the habitat health (Table 5) at each site I surveyed. The rubric for potential threats, (Table 6) was based on the five most common threats to EMR: road mortality, poaching, exposure to humans, predation and

Table 5

Habitat Assessment Rubric

Habitat Health Attributes	Score	Description
Open Canopy	1	No open canopy, often closed off by successional woody growth
	2	Open canopy with no cover
	3	Canopy open with cover nearby
Proximity to Water	1	Wetlands disappearing due to succession or human intervention.
	2	Flooding or drought has been frequent in this area. Water has been diverted.
	3	Wetland is healthy, no flooding or diverting of natural water sources to habitat
Dense Ground Cover	1	All dense ground cover destroyed on property
	2	Only small pockets of dense ground cover
	3	Dense ground cover near basking areas and hibernation areas.
Hibernaculum	1	Abundant crayfish holes, tree roots or sphagnum moss that can be used for hibernating.
	2	Crayfish burrows are few or separated by agricultural property or road.
	3	No obvious signs of crayfish or nearby hibernacula that can be used by EMR.
Size and Connectivity	1	Small, isolated habitat 20 acres or less.
	2	Larger than 30 acres but may have connectivity issues.
	3	50 acres or more with connectivity to seasonal habitat needs.

Table 6

Potential Threats Rubric

Potential Threats	Score	Description
Road Mortality	1	Busy road bisecting habitat.
	2	Quiet dirt road bisecting habitat.
	3	No roads bisecting habitat or the only roads are outside property.
Poaching	1	Evidence of hunting or poaching has been seen on the property.
	2	Habitat is near roads, people may be allowed to hike but it is carefully monitored by management.
	3	No signs of hunting or poaching on site. Site is hidden from view for most people.
Exposure to Humans	1	Foot traffic or even off-road vehicles allowed near or on property. See trash throughout habitat.
	2	Property is near roads but doesn’t seem to have people access it very often. See very little trash around habitat.
	3	No homes nearby, no roads most of the habitat is restricted access.
Habitat Destruction or Succession	1	No active management. No control of invasive plants or succession of woody plants.
	2	Habitat maintained but chances of chemical run-off from agriculture and homes are extremely close.
	3	Property is maintained and protected from future destruction. Invasive plant species are controlled

habitat destruction. A rubric was also developed to determine the conservation strategies of each site based on the five criteria for a good conservation strategy (Table 7). The rubric included many of the important regulations and suggestions in the CCAA for EMR. Each criterion for the three rubrics were scored qualitatively on a scale from 1-3 (1=poor or low quality, 2=baseline or satisfactory and 3=high quality). If no information was available about that particular criteria at each site, it was labeled as undetermined.

Results

During the two years and almost 200 hours of surveying (Table 8), a wide range of habitat health was seen. Sites were of varying sizes (Table 9) in acreage and had different levels of management.

Table 8

Calculated Hours at Each Site

Sites	Total Hours[a]
DWP	47.1
INB	12.8
BNC	98.2
BTF	45.1
MLB	18.8
TOTAL	**197.0**

[a] Depicts hours I personally spent actively searching for EMR at each site.

Table 7

Conservation Strategies Rubric

Conservation strategies	Score	Description
Management Plans	1	No goals for management. Either does no management of habitat or just every few years.
	2	Goals but not working directly with CCAA guidelines and lack of funding to accomplish goals
	3	Clear goals following CCAA guidelines and staying on target with goals each season.
Habitat Complexities	1	Invasive plant species allowed to create monocultures throughout property. No property management of habitats.
	2	Management only towards safety and concerns of EMR habitat.
	3	Work to keep natural complexities of habitat structure for numerous animals not just EMR.
Management to Maintain Natural State	1	No management at all. Much of site no longer has natural habitat look. local species have been undermined by invasive species.
	2	Have plan but not maintaining all the habitat. Allowing growth of unnatural (invasive plants) throughout property.
	3	Property has been included in CCAA for protecting EMR and other species. Uses CCAA schedule and plans for protecting all habitat.
Management of Water Levels	1	Small, isolated habitat 20 acres or less.
	2	Larger than 30 acres but may have connectivity issues.
	3	50 acres or more with connectivity to seasonal habitat needs.
Hibernaculum Management	1	Allowing succession or human encroachment into hibernaculum area.
	2	Any loss of hibernacula at all.
	3	No alteration of hibernaculum.

Table 9

Acreage of the Five Historical Research Sites[a]

Research Sites	Acreage
DWP	40
INB	95
BNC	159
BTF	65
MLB	109

[a] Five sites I surveyed and total acreage owned by each organization

DWP Assessment

Based on the results of the three rubrics, DWP received a high overall score. Although DWP is only 40 acres in size, its other habitat assets were high (Table 10). A lower potential threat assessment (Table 11) was given due to its high threat of road mortality. Before my research began the last two EMR sightings were both road kills. Poaching is also a potential issue because evidence of hunting and dumping of poached animals has occurred at this site. The lowest threat at this site was habitat destruction. A perfect conservation assessment (Table 12) was determined using the rubric. Its strongest area was a detailed management plan with lucid goals because DWP works within the CCAA in all aspects of habitat management.

INB Assessment

Based on the first rubric, INB received a relatively low assessment for habitat (Table 10). Its best asset was its size but many of the other categories were poor. Its highest potential threat was habitat destruction, though not due directly to human causes. Instead, the destruction of ideal habitat is overgrowth and invasive plants

Table 10

Habitat Assessment

Sites	Open Canopy	Dense Ground Cover	Proximity to Water	Hibernaculum	Size and Connectivity	Total	%
DWP	3	3	3	3	2	14	93.3
INB	3	2	2	U	3	10	73.3
BNC	3	3	2	2	3	13	86.7
BTF	2	2	3	3	2	12	80.0
MLB	2	1	3	2	2	10	66.7

Table 11

Potential Threats Assessment

Sites	Road Mortality	Poaching	Habitat Destruction	Predation	Exposure to Humans	Total	%
DWP	2	1	3	U	2	8	66.7
INB	3	3	1	U	3	10	83.3
BNC	2	2	3	U	2	9	75.0
BTF	2	U	1	U	2	5	58.3
MLB	3	U	1	U	2	6	66.7

Table 12

Conservation Assessment

Sites	Detailed Management Plan with Goals	Plan Keeps Habitat Complexities	Maintain *Ecosystems in Natural State	Management Water Levels	Clear Plan for Hibernacula Management	Total	%
DWP	3	3	3	3	3	15	100.0
INB	1	1	1	1	1	5	33.3
BNC	3	3	3	3	3	15	100.0
BTF	2	1	2	2	1	8	53.3
MLB	1	1	2	2	1	7	46.7

*Prairie, Bog, Woodland, and Peat

creating monocultures and woodlands creeping into the open canopy habitat. The lowest threat was road mortality since no roads come near this property. There are no strong areas within the conservation of the property according to my assessment rubric. At this time, no active management is occurring in any way. Based on the results of the three rubrics INB received a low overall assessment.

BNC Assessment

My largest site was BNC with a total of 159 acres (Table 9). Based on the results of the first rubric BNC received a high habitat assessment (Table 10) especially due to size and connectivity, open canopy and ground cover. The weakest aspect of the habitat assessment at this site was hibernacula, basically, it is not known exactly where the EMR hibernate at this site. I have seen numerous crayfish holes throughout the fen. The highest potential threat was road mortality, two EMR were found within 100 meters of the dirt road on both sides (Table 11). The busy road can also be a threat to the EMR since habitat is found on both sides of the road. The lowest threat would be habitat destruction, BNC has many facets to care for and protect all the ecosystems at this site, including a local volunteer steward that keeps an eye on the property almost daily. A perfect conservation assessment was determined using the rubric (Table 12). Its strongest area was maintaining the ecosystems in their near-natural state. When both DWP and BNC have controlled burns or take out invasive plants they plan very carefully to increase biodiversity. Based on the results of the three rubrics BNC received a high overall assessment.

BTF Assessment

The weakest habitat asset for BTF is its size (Table 9). Although 65 acres sounds like a lot, it is closed off by roads and warehouses, making the habitat area difficult to expand. Its highest potential threat was habitat destruction due to the occurrence of succession and invasive plant species within the open canopy zones (Table 11). Signs placed by the organization that owns the property help protect the area as well as the dense trees all along the outskirts. Very little active management occurs at this site therefore, the conservation assessment was low (Table 12). Other than some very basic management of signs and a bridge, this site has received only a small amount of conservation attention. Based on the results of the three rubrics BTF received a somewhat low overall assessment.

MLB Assessment

MLB includes a kettle bog and its surrounding habitats, 109 acres total (Table 9). All around the bog are open woodlands and a few homes. Based on the results of the habitat assessment rubric, MLB received a low score (Table 10). This site's best habitat asset was proximity to water, due to the simple fact that it is a kettle bog. The weakest aspect was open canopy because of the overgrowth along the forest and the edges of the bog. Buckthorn and berry plants grow thick along the boardwalk and the wooded areas are also losing their early successional habitat near the bog. Most of the wooded areas are thick and filled with undergrowth. A potential threat assessment total was very low for the bog using the rubric (Table 11). Its highest potential threat was habitat destruction both from the garbage dumping in the past and invasive species takeover currently occurring. Its strongest area in conservation management was water management because this particular bog is unique and protected. Through protecting the bog, water levels are also protected. Overall habitat complexities are not a major part of any management plan here, therefore this is the weakest part of the habitat's conservation.

Discussion

This study was able to provide information about current habitat threats observed at each of the five historical EMR sites during the two years of field research. Through visual surveys, potential dangers and conservation actions already occurring at each site were determined. Based on these ecological studies, habitat management methods can be suggested for ecosystem restoration. Some of these sites were easier to enter than others, some sites have areas that cannot be directly surveyed. However, field surveys suggest that each historic site has suitable habitat for the EMR and other important plant and animal species.

DWP and BNC, both scored much higher than the other three sites for their habitat assessment (93.3% and 86.7% respectively) (Table 10). DWP is a smaller property (40 acres) than BNC (159 acres), but crayfish holes for hibernacula were abundant. Invasive species are creating lower habitat health at both MLB and INB by decreasing open canopy. Many of these plants also produce large monocultures that close off connectivity and hibernaculums. Therefore, MLB and INB both scored the lowest for habitat assessment in BC (66.7% and 73.3%) (Table 10). BTF scored 80.0% because of its proximity to DWP and the large number of crayfish holes observed (Table 10).

Both DWP and BNC have roads that transect the habitats where EMR are most active providing the most danger. DWP received 66.7% (Table 11) in potential threat assessment due to poaching evidence and road dangers. BNC received 75% and INB received 83.3% (Table 11) in potential threat assessment due to fewer threats seen at these sites, INB is protected from most anthropogenic threats because of its more isolated location. INB has no roads or easily accessible trails into the habitat and it is considered a larger site at 95 acres (Table 9).

The INB and MLB sites have little management occurring and therefore, received the lowest conservation assessments (Figure 7). Succession, invasive species, and some loss of biodiversity have occurred at these sites. Overall these sites still have good habitat for the EMR and they offer a buffer between the best habitat and roads or homes.

Two of the properties, DWP and BNC are well managed and both received 100% in their conservation assessments (Table 12). The organizations that own these two sites actively control invasive species and woody succession with safe procedures such as

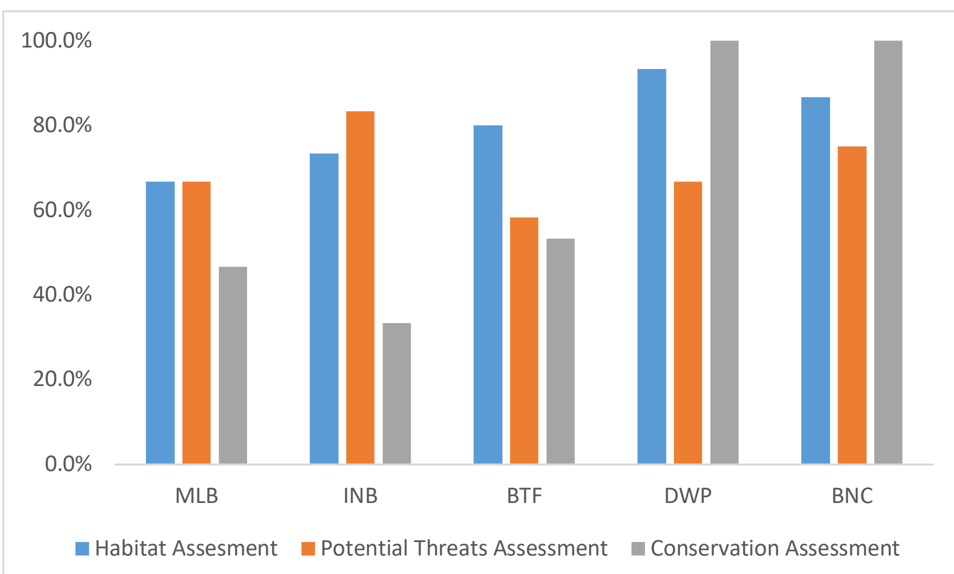

Figure 7. Comparison between sites.

controlled burns that are utilized only during EMR hibernation periods. Additionally, they carefully monitor any personnel that actively maintain these sites. Both sites intentionally collaborate with neighbors encouraging local residents to live peacefully with EMR and other important species.

After comparing all five properties using the three assessments, Figure 7 suggests that DWP and BNC are the healthiest and best-preserved sites and thus offer the healthiest and safest overall habitats for EMR to populate. However, habitat is still available yet more limited for important species such as EMR at each of these sites. What brings down the scoring of the properties in BC is poor conservation planning by MLB, INB and BTF all under 54% (Table 12 and Figure 7).

Conclusion

Two areas on the rubrics were relatively unknown showing that these are weaknesses to be further studied. The first of these relative unknowns was hibernaculum. Although at a few of the sites, crayfish holes were common, understanding if EMR use them will take a focused study on that topic. Determining where EMR hibernate is difficult since they use crayfish holes and need the water table to be high enough to prevent desiccation during hibernation. It would be best to determine which areas on the property are most often used during winter ingress so those specific sites can also be protected, especially with water level issues being such an important determinant to survival during hibernation for EMRs. The second area of unknown was predation. Evidence of predators was seen at DWP, but this aspect of EMR life was not specifically studied at each property. Trail Cameras and live traps could be set up to determine predators that live within the properties.

Each of these properties have individuals and organizations working to protect at least some aspect of the environment. But as humans continue to encroach on Michigan's natural areas more research is crucial.

CHAPTER 4

GENETIC DIVERSITY OF THE EASTERN MASSASAUGA (*SISTRURUS CATENATUS*) IN SOUTHWEST MICHIGAN (BERRIEN AND VAN BUREN COUNTIES)

Introduction

The EMR is a small, robust pit viper currently found in nine states and the province of Ontario, Canada. Eastern massasauga rattlesnakes typically inhabit shallow wetlands with few trees. These habitats have experienced significant destruction by humans and as a result the current distribution of the EMR is a fraction of its historic distribution (Szymanski, 2015). Being both predator and prey makes the EMR an integral part of the ecological community. The EMR has been extirpated from much of its range in Michigan and even more so in the other states where it was once abundant.

In 2016 the EMR became listed by the USFWS as a threatened species (USFWS, 2016). Although Michigan is the last stronghold for the EMR, many populations have been left unstudied. For example, BC contains 8 known historical sites for the EMR and other anecdotal sites; nonetheless no formal studies have been conducted here to date (Michigan Natural Heritage Database, 2014). As a threatened species, it is crucial to manage the remaining populations of EMR when possible. Clear conservation goals should be set up to manage any species and one important area to guide conservation is genetic diversity (Moritz, 1994). There are many different aspects of genetic threats to look at and therefore, it is important to study as many as possible to increase our understanding of threatened and endangered species (Amos and Balmford, 2001; Sovic, 2019).

The EMR populations tend to be small and fragmented throughout their range (Szymanski, 2013; Greene, & Campbell, 1992). The cryptic nature of EMR leads to a lack of information about the population dynamics, so genetic studies can provide some of the missing information on their demographic patterns and genetic variations within isolated populations (Anderson et al., 2009; Gibbs et al., 1997l; Ray et al., 2013). Microsatellite DNA studies on EMR populations can prove useful for a variety of analyses at the population level (Gibbs et al., 1998). Numerous studies have occurred on the EMR using mitochondrial and microsatellite DNA in an effort to define local populations and management units (Gibbs et al., 1997; Kubatko et al., 2011; Ray et al., 2013; Sovic, Fries, & Gibbs, 2016; Sovic, Fries, Martin, & Gibbs, 2019).

One genetic analysis of the eastern massasauga demonstrated the existence of three geographic subunits (Ray et al., 2013). Using the NADH dehydrogenase subunit II (ND2) gene in the analysis of mitochondrial Deoxyribo Nucleic Acid (mtDNA), three distinct geographical haplogroups were identified as western, central, and eastern. A haplogroup is defined as a network of closely related haplotypes (a group of alleles inherited together) within a species, that share a common ancestor where the genetic sequence is only one or two base pair (bp) differences from each other. E.g. Haplotype 2, 3, 20, 17 are all only one bp different from haplotype 1. Haplotype 4 is one different from 17 so it is shown next to 17 but within haplogroup 1 (Figure 8). Between 1 and 5 there are four bp differences, but between 17 and 5 there are only three bp differences therefore 17 is between 1 and 5 but more closely related to haplogroup 1. Haplotype 5 is the center

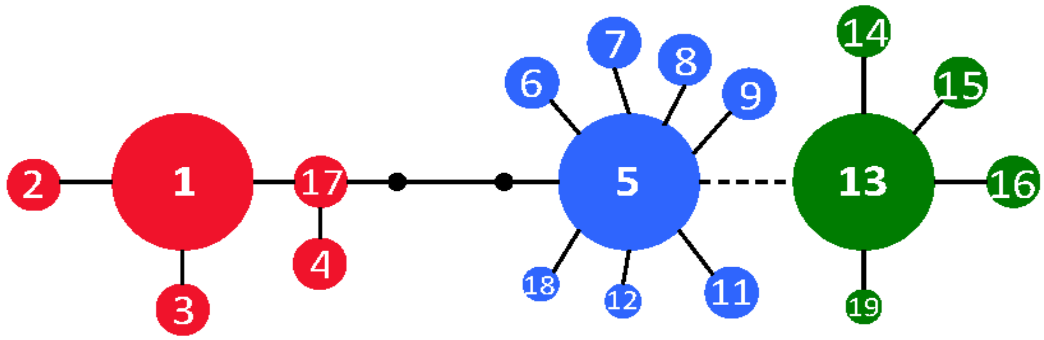

Figure 8. Eastern massasauga rattlesnake ND2 haplotypes in North America. Colors distinguish three ND2 geographic subunits or haplogroups (eastern ND2 subunit = green, central ND2 subunit = blue, western ND2 subunit =red). Small, medium and large circles represent sample size (l n=1, n=2-19, n=20+ respectively). A 3-base-pair deletion separates haplotypes 5 and 13 (dashed line). Figure modified from Ray et al. 2013. Black dots depict two possible haplotypes that are not yet discovered.

of the next cluster of haplotypes because they all have only one bp differences between themselves and 5. (6, 7, 9, 18, 12, 11) and is more similar to 17 and 1 than 13 so it is in between 1 and 5.

Ray et al. (2013) performed genetic analysis of blood samples from 34 unique locations throughout the full range of the EMR. The historic populations of EMR include 165 counties of which only 28 individual counties were tested (Figure 9). From these 34 unique locations, 18 haplotypes were discovered and clustered into three geographic groups or haplogroups (Figure 9). Although this study included fewer than 20% of the historic populations of EMR, it was exhaustive compared to previous studies on the genetics of EMR. Large regions within the EMR range failed to be included in the genetic testing, including regions in northern Indiana and southern Michigan (Figure 9).

Because BC is known to contain a number of historic populations, I wanted to determine how this county fit into the genetic puzzle of the EMR. To date, most EMR research has been concentrated in CC, Michigan and numerous counties further north and

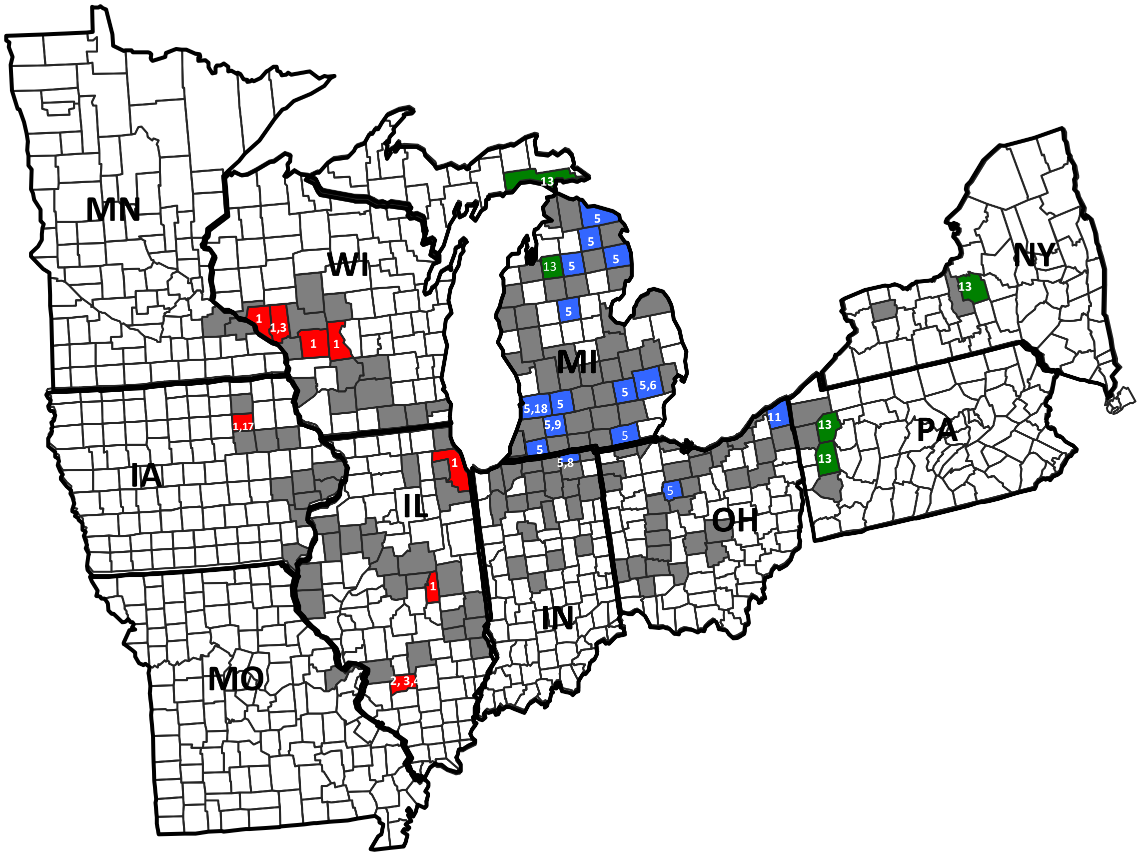

Figure 9. Historic distribution of eastern massasauga. Data compiled from state records and publications Syzmanski et al., 2015. Counties with verified records are shaded gray; counties from which samples for genetic analysis were obtained are shaded according to haplotype analysis from Ray et al., 2013 as follows western haplotypes (red), central (blue) and eastern haplotypes (green).

east of BC (Chiucchi, 2011; Gibbs et al., 2011; Ray et al., 2013; Szymanski et al., 2015). Eastern massasauga rattlesnakes tested in surrounding counties in Michigan have defined haplotypes within the central haplogroup (haplotype 5, with the addition of haplotypes 6-9, 11-12, 18) and the EMR tested in the Chicago area of Illinois and west, were considered to be in the western haplogroup (haplotype 1, with the addition of haplotypes 2-4, 17). I set out to study BC EMR populations and add clarity to the western and central haplogroups boundary, predicting that all of the snakes would be haplotype 5 or a close variation based on the known haplotypes in nearby counties (Figure 8). My results regarding genetic variations found in BC were compared to nearby Michigan, Indiana and Illinois counties EMR (Anderson et al., 2009; Ray et al., 2013).

Materials and Methods

Field Methods

Visual surveys were conducted for two years (2016 and 2017) at historical population sites in Southwest Michigan. A total of thirteen EMR were captured during these surveys, 2 captured in CC, 3 in Van Buren County (VB) and 8 EMR at sites in BC. In 2016 and 2017 I obtained a permit from Michigan DNR to capture and draw blood from each EMR discovered. In 2017 I also obtained a permit from USFWS to handle EMR.

All EMR were handled using standard protocol with approval by Andrews University Institutional Animal Care and Use Committee (IACUC). Each EMR was processed on site. The EMR was tubed using a clear plastic "snake tube". Once two-thirds of the snake's body was inside, an assistant held the snake securely while I drew blood from the caudal vein using a sterile 1 cc syringe with a 25 gauge 5/8" needle. A few drops of blood were placed into a micro-centrifuge tube containing 10 microliters (μl) of extraction buffer (0.5 M Tris, 0.25% Ethylenediaminetetraacetic Acid, and 2.5% SDS) (Burbrink and Castoe, 2009). Samples were stored at -86° C until DNA extraction.

Lab Methods

DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Inc., Valencia, CA) following the manufacturer's protocol for nucleated blood. These DNA extracts were the templates for the next step in the process: amplification of the mitochondrial ND2 gene by polymerase chain reaction (PCR). All PCR procedures were performed as described by Ray et al. (2013) to enable subsequent comparison with their data. Forward and reverse primers were added to PCR amplification mixes (Anderson et al., 2009; Ray et al., 2013). These PCR reaction mixes consisted of 36.5 µl deionized water, 5 µl 10 X DreamTaq Green Buffer, 5 µl 2 mM dNTP mix, 1.25 µl CE2330 primer (5'-CTA ATA AAG CTT TCG GGC CCA TAC-3'), 1.25 µl CE2331 primer (5'-TTC TAC TTA AGG CTT TGA AGG C-3'), 1 µl template DNA, and 0.25 µl DreamTaq DNA polymerase. A PCR was performed in a Bio-Rad T100 thermal cycler with a cycle consisting of a 3-min initial denaturing step at 95° C, followed by 40 cycles of 30-secs at 95° C, 30-secs at 50° C, and 90-sec at 72° C, and a final elongation step of 7-min at 72° C.

The resulting amplicons were resolved on a 1% agarose gel. Relevant bands, which were 1000 bp in size, were excised with a clean scalpel and purified using the QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's protocol. Each sample was sequenced (GenScript) using both the CE2330 primer and an internal primer (L5238s: 5'-ACT TGA CAG AAA ATT GCC CCC-3'). The sequences obtained were checked for quality and aligned with previously identified ND2 haplotype sequences (Ray et al., 2013) using Unipro UGENE, distributed under the terms of the General Public License. Variable bps were confirmed visually analyzing chromatogram files for signal strength and quality of bp calls. Haplotypes were identified by visual inspection of aligned sequences. The program UGene was used to compare each of the sequences of my EMR with the 18 haplotypes identified by Ray et al. (2013). Each identified sequence was placed in corresponding haplotypes as discussed in Ray et al. (2013).

Results

My samples of EMR came from the Southwest corner of Michigan from three counties (Figure 10). The CC site, previously used by Ray et al. (2013) was used as my control site. By processing EMR from CC, I could verify my procedures in the lab. I used two EMR from CC; Ray et al. (2013) also had two blood samples from this same locale.

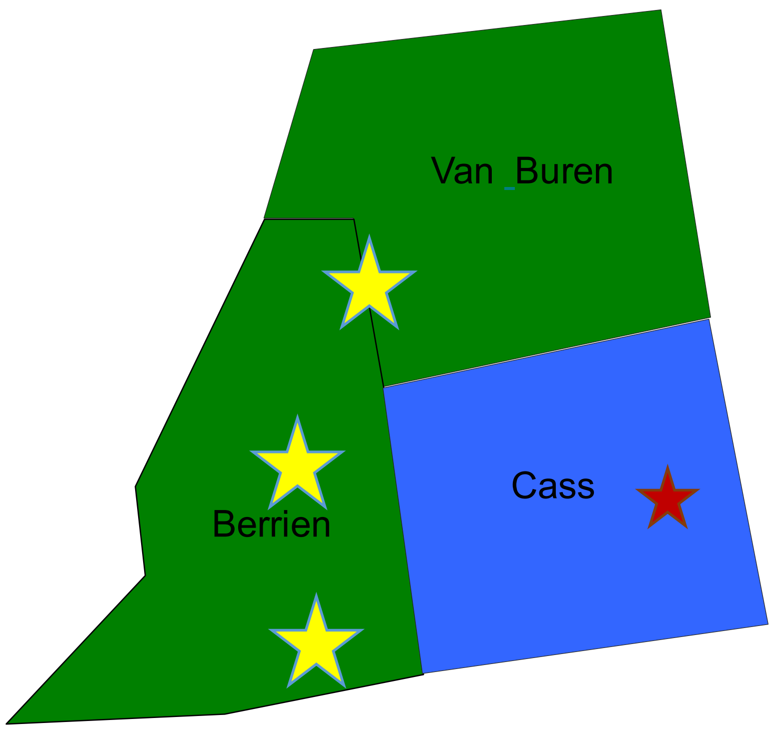

Figure 10. Map of Michigan counties where research took place. Cass County with red star depicts site where "control" snakes were found. Berrien and Van Buren Counties with yellow stars show the 3 sites where EMR were found.

The remaining three sites in my study were primarily in BC with one site crossing over into VB.

After comparing each mtDNA result of the 13 snakes I ran ND2 mtDNA tests on, the two EMR from Ed Lowe foundation in CC, were both determined to be Haplotype 5. The EMR from INB, in BC, Michigan was also determined to be haplotype 5 (all haplotypes in the central haplogroup are depicted as blue circles in my adapted figure of circles). The EMR found in the smaller 40-acre site in the southern part of BC, DWP, were determined to have one of two different haplotypes. Two EMR were only one bp different from the haplogroup 1 (Table 13). Yet, the EMR were different from all previously discovered haplotypes, making it a newly discovered haplotype 20 in the western haplogroup. The other four EMR captured at this site were also found to be a newly determined haplotype 21 (Appendix B shows the haplotype sequences). They all had identical bps with only one difference from the central haplogroup 5. These were also three bp different than the western haplogroup placing these snakes into a unique position discussed by Ray et al. (2013) (Figure 11 - the cluster circles, adapted from Ray et al., 2013). At BNC, which borders both BC and VB, I captured and tested four EMR. Their haplotype was verified as 13, which is considered to be an eastern haplotype, depicted as green in figures. All previously discovered eastern haplotypes found during Ray et al. (2013) are in northern Michigan, Canada and the more eastern portions of the EMR range. Eastern haplotypes contain a three-base deletion from bp 576-578. All western and central haplotypes have a tandem repeat of CCTCCT while the eastern haplotypes only have one copy “CCT”. When I analyzed each haplotype for my research, it was determined that haplotype 8 was actually two bp different than 5, not one bp as depicted in Ray et al. (2013) (See Figure 11).

Table 13

Location of Variable Nucleotides in Sequences

Code	haplotype	140	215	235	258	292	337	364	367	370	376	379	500	523	550	579	580	581	586	693	842	853	860	876	SITE
SICA 1	1	A	C	A	C	C	T	C	G	G	G	A	A	T	C	C	C	T	A	A	C	T	G	T	
AU007	20	A	C	A	C	C	T	C	G	G	G	A	A	T	C	C	C	T	A	A	A	T	G	T	DWP
AU003	20	A	C	A	C	C	T	C	G	G	G	A	A	T	C	C	C	T	A	A	A	T	G	T	DWP
SICA 2	2	A	C	A	C	T	T	C	G	G	G	A	A	T	C	C	C	T	A	A	C	T	G	T	
SICA 3	3	A	C	A	C	C	T	C	G	G	G	A	A	T	C	C	C	T	G	A	C	T	G	T	
SICA 4	4	A	C	A	C	C	T	G	G	G	G	A	A	T	C	C	C	T	A	G	C	T	G	T	
SICA 17	17	A	C	A	C	C	T	G	G	G	G	A	A	T	C	C	C	T	A	A	C	T	G	T	
SICA 5	5	A	T	A	C	C	T	G	G	G	G	T	A	T	C	C	C	T	A	A	C	C	G	T	
C-2	5	A	T	A	C	C	T	G	G	G	G	T	A	T	C	C	C	T	A	A	C	C	G	T	EDL
C-4	5	A	T	A	C	C	T	G	G	G	G	T	A	T	C	C	C	T	A	A	C	C	G	T	EDL
AU010	5	A	T	A	C	C	T	G	G	G	G	T	A	T	C	C	C	T	A	A	C	C	G	T	INB
AU008	21	A	T	A	C	C	T	G	G	G	G	T	A	T	C	C	C	T	A	A	C	T	G	T	DWP
AU009	21	A	T	A	C	C	T	G	G	G	G	T	A	T	C	C	C	T	A	A	C	T	G	T	DWP
AU001	21	A	T	A	C	C	T	G	G	G	G	T	A	T	C	C	C	T	A	A	C	T	G	T	DWP
AU005	21	A	T	A	C	C	T	G	G	G	G	T	A	T	C	C	C	T	A	A	C	T	G	T	DWP
SICA 18	18	A	T	A	C	C	T	G	G	G	G	T	A	T	C	C	C	T	A	A	C	C	G	C	
SICA 6	6	A	T	A	C	C	T	G	A	G	G	T	A	T	C	C	C	T	A	A	C	C	G	T	
SICA 7	7	A	T	A	C	C	T	G	G	G	A	T	A	T	C	C	C	T	A	A	C	C	G	T	
SICA 8	8	A	T	G	C	C	T	G	G	A	G	T	A	T	C	C	C	T	A	A	C	C	G	T	
SICA 9	9	A	T	A	C	C	C	G	G	G	G	T	A	T	C	C	C	T	A	A	C	C	G	T	
SICA 11	11	T	T	A	C	C	T	G	G	G	G	T	A	T	C	C	C	T	A	A	C	C	G	T	
SICA 12	12	A	T	A	C	C	T	G	G	G	G	T	A	C	C	C	C	T	A	A	C	C	G	T	
SICA 13	13	A	T	A	C	C	T	G	G	G	G	T	A	T	C	-	-	-	A	A	C	C	G	T	
AU002	13	A	T	A	C	C	T	G	G	G	G	T	A	T	C	-	-	-	A	A	C	C	G	T	BNC
AU004	13	A	T	A	C	C	T	G	G	G	G	T	A	T	C	-	-	-	A	A	C	C	G	T	BNC
AU006	13	A	T	A	C	C	T	G	G	G	G	T	A	T	C	-	-	-	A	A	C	C	G	T	BNC
AU011	13	A	T	A	C	C	T	G	G	G	G	T	A	T	C	-	-	-	A	A	C	C	G	T	BNC
SICA 14	14	A	T	A	T	C	T	G	G	G	G	T	A	T	C	-	-	-	A	A	C	C	G	T	
SICA 15	15	A	T	A	C	C	T	G	G	G	G	C	A	T	C	-	-	-	A	A	C	C	G	T	
SICA 15b	15	A	T	A	C	C	T	G	G	G	G	C	A	T	C	-	-	-	A	A	C	C	G	T	
SICA 16	16	A	T	A	C	C	T	G	G	G	G	T	A	T	C	-	-	-	A	A	C	C	A	T	
SICA19	19	A	T	A	C	C	T	G	G	G	G	T	A	T	T	-	-	-	A	A	C	C	G	T	

The mtDNA results from Berrien & Van Buren EMRs (n=11) + 2 from Cass Co. (Ed Lowe site as a control) resulted in 4 haplotypes representing all three haplogroups. Each bp highlighted in yellow is a variable nucleotide.

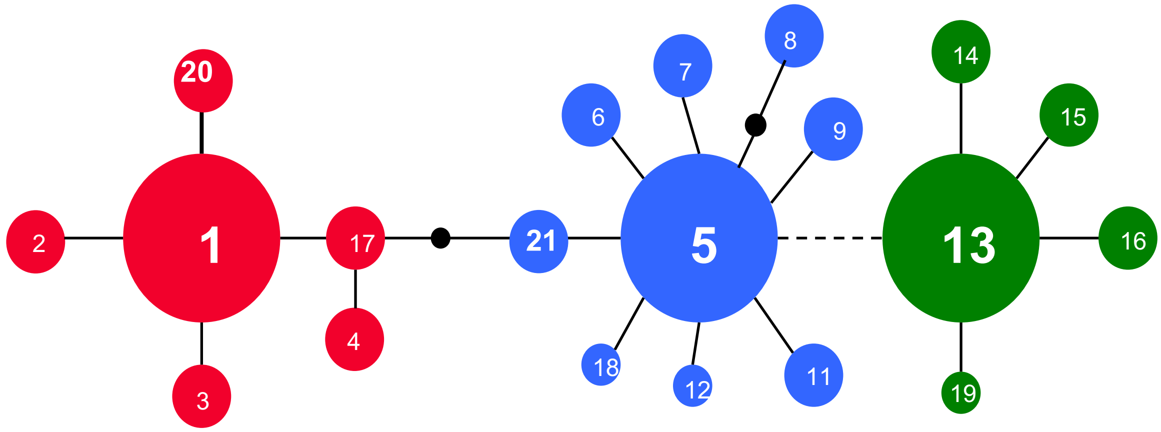

Figure 11. Network of EMR ND2 haplotypes in North America including BC. Dots denote unrepresented haplotypes. Berrien & Van Buren EMR (n=11) + 2 from CC resulted in 4 haplotypes representing all three ND2 subunits. DWP - 2 different haplotypes (both new; 20 & 21) from two different subunits (1 & 5) Colors represent three geographic ND2 subunits (eastern ND2 subunit = green, central ND2 subunit = blue, western ND2 subunit = red). (Adapted from Ray et al., 2013).

I identified four different haplotypes representing all three haplogroups as described in previous research (Ray et al., 2013). The 11 EMR were found at three historical sites in Berrien and on the edge of Van Buren Counties.

Discussion

While previous research demonstrated three distinct geographic ND2 subunits (namely western, central and eastern), my local populations encompassed all three ND2 subunits. My samplings came from three distinct populations all within BC. A corner of an adjacent county was also incorporated into one of the populations, VB. My fourth population, used as a control, was in CC these two snakes were determined to be the same central haplotype as previously tested in Ray et al. (2013), haplotype 5.

Although my sample size was only 11 snakes in two counties, my sample size is similar to the individual site sample size in Ray et al. (2013). Figure 12 is a map with

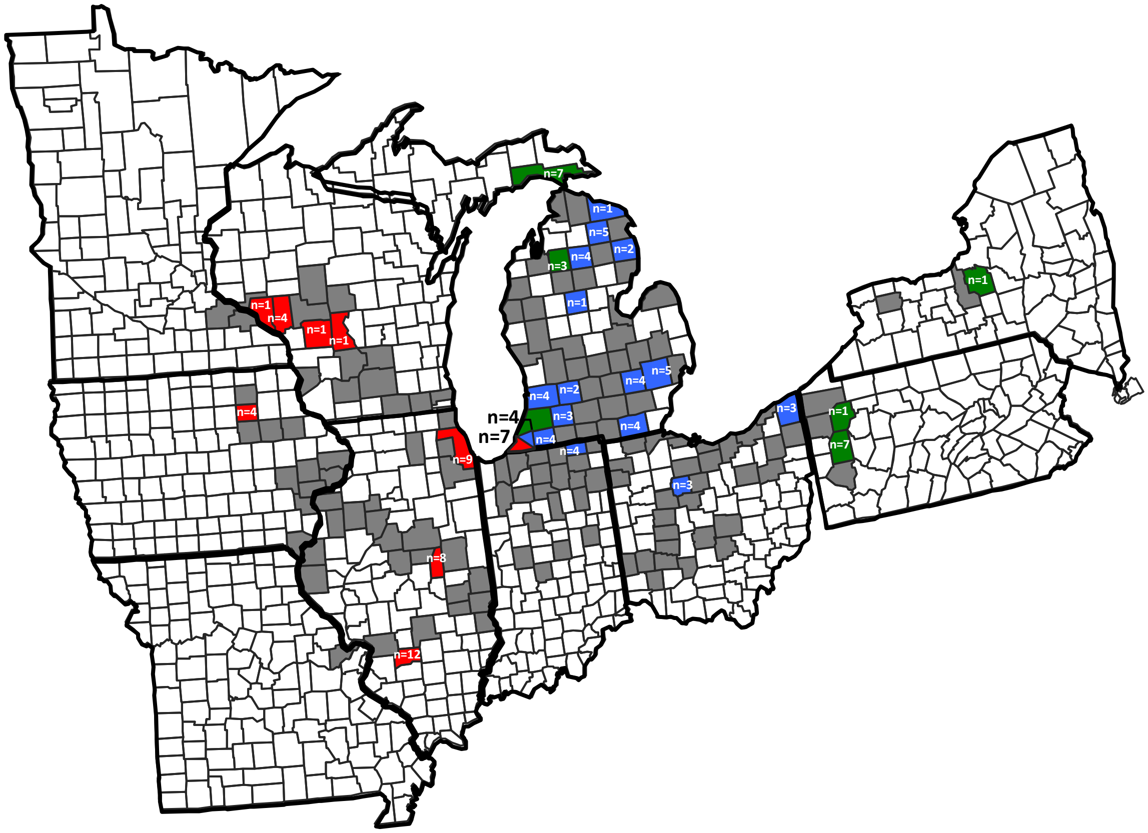

Figure 12. Map of DNA samples used in EMR mtDNA testing. My research added two samples to the CC samples which were previously (n=2). The eleven snakes found in Berrien and Van Buren Counties are n=7 and n=4 respectively. (The print is enlarged and in black to denote the research sites).

number of samples n= from each site in both Ray et al (2013) and my research. I expected to determine the boundary between western and central management units. Instead a discovery of western, central, and eastern haplotypes all within Berrien and Van Buren counties in southwest Michigan challenges the current ideas of EMR distribution and history (Figure 13). This diversity no longer fits the criteria for separate management units (Ray et al., 2013). The discovery of all three haplogroups within one county was unexpected. Eastern haplotypes found during Ray et al. (2013) were in northern Michigan, Canada and the more eastern portions of the EMR range yet four EMR were determined to be haplotype 13.

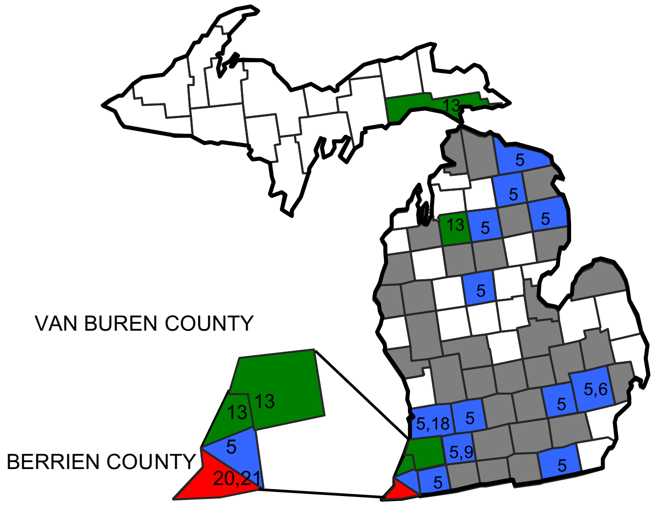

Figure 13. Map highlighting haplotypes in Michigan with enlarged insert of research results. The two 13's illustrate that EMR's with the eastern haplotype 13 were found in both counties. (This occurred at the site that crosses the county line.). The 5 represents another site where only one snake was found, it was haplotype 5. 20, and 21 were both found at another site, this site therefore has both central and western haplotypes.

My findings show a more locally diverse population in Southwest Michigan than any other known populations at this time using mtDNA. Perhaps my area is a center of diversity for the EMR and the eastern haplogroup is a subset of this diversity. Previous studies have assumed the need to manage the EMR geographically, assuming phylogeographic structure across its range (Gibbs and Chiucchi, 2012; Ray et al., 2013). More recently Sovic et al. (2019) have shown that there are numerous aspects of the EMR to look at before assuming the need to set up geographically separate management units (Sovic et al., 2019). My small study in southwest Michigan seems to uphold this idea as well. Continuing to study genetic information and historical demographic processes can help to piece together the best management practices for robust populations of EMR. To preserve EMR populations, it is important to understand that genetic drift has a significant negative effect on the isolated populations of EMR (Sovic et al., 2019).

Although Sovic et al. (2019) made some interesting points in their research, Michigan and Indiana were not included in the range wide analysis. My results depict populations with possible high levels of genetic variation and will be important to the future of the EMR. Further studies should be done in this area to compare southwest Michigan with surrounding populations that have not yet been genetically tested.

CHAPTER 5

CONCLUSION

The overall objectives of this research were to (1) determine continued EMR presence using a variety of validated and novel survey techniques at selected historic population sites in BC, (2) assess the remaining EMR habitat (habitat health, general threats, and conservation efforts) occurring at each visited historic population site in BC, and (3) determine genetic relatedness of BC EMR with other populations.

Eastern massasauga rattlesnakes continue to decrease in populations and numbers and are listed as a threatened species by USFWS (2016). Therefore, it is crucial to determine where all the extant populations exist. This information can aid the state of Michigan in protection of the species. My research conclusively determined EMR presence at three of the five historical sites surveyed in BC. Additionally, reproductive populations were discovered at two of the three sites. At the two sites where I found no evidence of EMR presence it has now been over 24 years since any EMRs have been seen. However, due to the cryptic nature of EMR and difficulty in determining which areas at these historical sites EMRs are likely to be found, it is vital that more studies be conducted before determining if the EMR population on these two sites should be considered extirpated. This will require a large investment of time; my research indicates that at least 38.2 person-hours are needed to detect a single snake. Additionally, large "bio blitzes" should be coordinated at each of the sites.

Habitat management has been and continues to be a challenge at each of the historical sites. The primary threats to EMR and other species at these sites are human intrusion, shrub encroachment, woodland succession and invasive species. Two sites are well managed and the properties have diverse microhabitats for EMR matching their seasonal needs. The owners control invasive species and possible succession with safe controlled burns during EMR hibernation periods. Additionally, these sites maintain a collaborative relationship with neighbors encouraging the local residents to live peacefully with EMRs and other important species. However, both of these sites do have roads that transect the habitat where EMRs are most active and there is also evidence of poaching and lots of garbage along the roadside of one of these sites. These are common issues that need to be continually addressed. Eastern massasauga rattlesnakes were found at both of these sites.

At another site there are no issues with human intrusion but scrub shrub encroachment, woodland succession, and invasive species are destroying the ideal habitat for EMR. It will be an expensive and time-consuming endeavor to manage these issues. However, this site and the other historical sites need ongoing strategic management to help produce ideal habitats for future generations of EMR as well as other herpetofauna. One EMR was discovered at this site.

The genetic diversity discovered during my research supports the idea that these population sites are crucial to the future of EMR. Genetic diversity adds vigor and overall survivability to populations. The haplotype diversity discovered during my research has not been encountered in any other county where EMR have been studied. DWP has the most diverse population genetically, haplotypes designated to multiple management units (western and central) were detected at this site. Although it is my smallest site, only 40 acres, a four bp distance between the haplotypes of EMR have been determined at DWP. Therefore, I believe that DWP is a very important habitat to protect.

Haplotype 13, which is associated with the eastern management unit was detected in all my snakes from BNC. This further highlights the importance of BC to EMR conservation. Numerous Eastern box turtles, also a threatened species, were found at BNC. Since this property is ideal for both these herpetofauna and it is already protected and managed, this site should also be considered important to EMR survival.

The total number of haplotypes discovered in BC was four, representing all three management units or haplogroups. Previously these management units have been thought to be genetically separated. Locating all haplogroups within one county could modify the picture of the known geographic variation previously thought to exist (Ray et al., 2013). In light of this new information, a new interpretation of gene flow and historic dispersal should be considered. Possibly BC is a relic of all the mtDNA haplogroups making it the ancestral foundation for EMR. From here EMR have radiated out, colonizing north, east and west. In conclusion, the research in BC should give rise to more thorough genetic research in EMR populations throughout their range.

APPENDIX A

PROTOCOL

Working Protocol

A) Any minor injuries, including bites or scratches by nonvenomous or venomous animals, must be reported within 24 hours to the project PI (Dr. Daniel Gonzalez-Socoloske).

B) In the event of envenomation in the field:
   i) **Remain calm**. Secure or release the snake if possible.
   ii) Note the species of snake that has bitten you and its approximate size (length). Also take note of the time. Massasaugas are the only venomous snakes in Michigan so identification should not be a problem.
   iii) **Immediately notify any companion with you that you have been bitten.** If you are separated from your companion(s) or alone, proceed to the next steps.
   iv) **Dial 911 by cell phone**. Inform the operator that a venomous snake has bitten you. Arrange for emergency transportation to the nearest hospital.
      i) OR, alternatively, have someone at drive you immediately to the nearest hospital.
   v) Once your transport to or arrival at the hospital is assured, have someone contact Daniel Gonzalez-Socoloske (269-408-6892) or another faculty member of the Department of Biology to inform him or her of the incident.

Hygiene Protocol

1. Before leaving each site, wash off as much of the mud/dirt on equipment and gear and remove any vegetation or detritus attached to gear by shaking and hand picking.
2. Do all sterilizing well away from streams or ponds.
3. Bring bucket with two gallons (eight quarts) of clear water and 12 capfuls (6 tablespoons) of bleach to each site for cleaning of all equipment.
4. Dip and rotate traps or snake tongs and other equipment that comes in contact with the animal or the local substrate) in solution, shake off, open, and lay out to dry.
5. Clean shoes or boots by dipping scrub brush in solution and scrubbing entire outer surface, shake off and let dry in sun.
6. Scrub waders in a similar fashion to boots and shoes.
7. Save sterilization solution in a sealable container between uses. Discard after every couple of trips by disposing of on asphalt, cement or hard roadbed, well away from any water bodies.
8. When possible, allow gear to dry completely before using at future sites (Kingsbury, 2012).

APPENDIX B

NEW DNA SEQUENCES

Haplotype Sequence-20: EMR-AU007, 003

CATCTCAAAACCCCACCACCCCCGGGCCACAGAAGCAGCAACAAAGTACTTC
CTTACACAGACTCTAGCCTCCACAGCTATCCTTTTTGCAGCAACAATAAACGC
ACTTAATTCCTCAAACTGAGAAATCACTCTCACTACAGAAACCACAACAATA
AAAATCATTACACTAGCCCTAATAATAAAAATAGCCGCAGCTCCTTTCCACTT
CTGATTACCAGAAGTGACACAGGGAGCCACAACACTAACAGCCCTAACAATC
CTAACTTGACAGAAAATTGCCCCCCTATCCATTCTTATAGCTAATCACAATAA
CACCAACCTAACAATCTTAATTTCATCTGCAATTTTGTCTATCCTAGTGGGGG
GGGTAGGGGGTTTAAATCAAACCCAACTACGAAAACTCATGGCCTTCTCATC
TATCGCCCACACAGGATGAATCCTTGCAACCATTACCCTAGCACCAAATATCT
CCATCCTTACCTTCCTAATCTATACAATAACTACCATCCCAATCTTTATTGCAC
TAAACACGTCATCAACAACAACCATTAAAGACTTAGGAGTCATATGAACCTC
CTCCCCATACCTAATGCTTATCACTTTAACCACTATTCTATCCCTAACTGGCCT
TCCACCCCTTACAGGCTTTATACCAAAATGATTAATTCTTAACAAAATAACCG
CCTTCAACCTAACTACAGAAGCCACCCTCATAGCTATAACCTCACTACCCAGT
CTATACATATATATCCGACTAACCTACATCCTAACCATAACGCTTTCCCCCCA
CACATCCACCACACAAATAAAATGACGAATCCCACACAAAAATCTCCCCCTA
TTACCAATTACCCTCGCTGCCATAACAACCTTTCTCCTGCCCATAACCCCGAC
CCTA

Haplotype Sequence-21: EMR-AU001, 005, 008, 009

CATCTCAAAACCCCACCACCCCCGGGCCACAGAAGCAGCAACAAAGTACTTC
CTTACACAGACTCTAGCCTCCACAGCTATCCTTTTTGCAGCAACAATAAACGC
ACTTAATTCCTCAAACTGAGAAATCACTCTCACTACAGAAACCACAACAATA
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CTGATTACCAGAAGTGACACAGGGAGCCACAACACTAACAGCCCTAACAATC
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TTACCAATTACCCTCGCTGCCATAACAACCTTTCTCCTGCCCATAACCCCGAC
CCTA

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